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Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

19th European Biomass Conference & Exhibition
Berlin, 6-10 June 2011

Combined silage treatment and enzymatic hydrolysis of energy grass as pretreatment method for 2nd generation bioethanol production.

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Pretreatment and enzymatic hydrolysis for conversion of lignocellulosic biomass to fermentable sugars is often the most expensive steps in 2nd generation bioethanol production. The presented work aimed at studying a low energy pretreatment method using silage treatment combined with enzymatic hydrolysis. Silage treatment is the anaerobic biological process where Lactic acid bacteria consume free soluble sugars in the biomass and produce lactic acid. The drop in pH prevents further biological degradation and thus conserves the biomass. The moist acidic conditions hydrolyze the biomass to a certain extent and facilitate better enzymatic conversion. The biomass used in the study was two species of high yielding energy grass (ca 15-17 tonnes DM/hectare) Hykor Festulolium and Achilles Festulolium provided by DLF TRIFOLIUM A/S. The fresh grass was dried to DM between 25-50%, cut to 2-4 cm, sprayed with and without industrial silage inoculums (LACTISIL Grass Plus and LACTISIL CCM from Christian Hansen), ensiled in plastic bags at 99% vacuum and disrupted at different times. After two weeks the production of lactic acid stopped at a concentration of around 8.0 mg/gDM, and the silage was fully conserved. The ensiled grass was hydrolysed by Cellic™ CTec2 enzymes from Novozymes, and the efficiency of the treatment was measured by both convertibility of cellulose, and ethanol yield in batch fermentations with *Saccharomyces Cerevisiae*. Changes in the microbial flora during silage treatment were investigated using DNA extraction and denaturing gradient gel electrophoresis (DGGE). The pretreatment method present a simpler, less energy intensive and cheaper possibility of producing 2nd generation bioethanol. Furthermore, the method can potentially be implemented at the biomass producers (the farmers) and used, at the same time, as a storage method for bioenergy-biomass.